

Amendments to the Claims:

The listing of claims will replace all prior versions, and listing of claims in the application.

1. (Original) A method for identifying essential and non-essential genes in a genome of a cell grown in non-selective conditions, said method comprising:
  - saturation mutagenesis of said genome by insertion mutagenesis, whereby an oligonucleotide sequence is inserted in the target regions of said genome such that a population of cells having at least 90% of said target regions insertionally mutated is obtained;
  - growing said population of cells under non-selective conditions to provide a non-selected sub-population of cells;
  - amplifying a target region from said non-selected sub-population of cells, using a first primer which hybridizes to a known first end of said target region, and a second primer which hybridizes to another known end of said target region, said first and second primers thereby constituting a first primer pair, giving rise to a first extension product, and a third primer which hybridizes to said oligonucleotide sequence, said third primer constituting a second primer pair with one said first or second primer, said second primer pair enabling the amplification of a second extension product; and
  - assessing for the presence or absence of said first and second extension product, whereby the presence of the first and second extension products is indicative of a non-essential gene, whereas the presence of the first extension product and the absence of the second extension product is indicative of an essential gene.
2. (Original) A method according to claim 1, wherein mutagenizing is performed with a transposable element.
3. (Original) A method according to claim 2, wherein said target DNA comprises a gene encoding a protein.

4. (Original) A method for functional analysis of a target region in a sequence of interest, said method comprising:

mutagenizing said target region by insertion of a sequence tag to provide a population of DNA molecules containing a sequence tag insertion in at least 90% of nucleotide positions in said target region;

introducing said population of mutagenized DNA molecules into host cells that express said sequence of interest;

subjecting a first aliquot of said host cells to at least one selective condition and a second aliquot to a non-selective condition to provide at least one selected and one non-selected aliquot;

amplifying said target region from said at least one selected and one non-selected aliquots, using a first primer hybridizing to said sequence tag and a second primer hybridizing to a known endpoint, said endpoint being characterized as an arbitrary unique sequence in said target DNA, to provide amplified DNA; and

resolving by gel electrophoresis said amplified DNA from said at least one selected and one non-selected aliquots into individual bands differing by size to identify the position of individual sequence tag insertions within said target region,

whereby differences between the presence or intensity of bands between said at least one selected and one non-selected aliquots are indicative that said sequence tag insertion causes a difference in response to said selective condition employed with said at least one selected aliquot resulting in the functional analysis of said target region.

5. (Original) A method according to claim 4, wherein mutagenizing comprises the steps of:

combining DNA comprising said target region with retroviral integrase and a first set of complementary oligonucleotide primers, said primers comprising (a) a recognition sequence for said retroviral integrase and (b) a sequence tag, wherein said retroviral integrase mediates the insertion of said first set of complementary oligonucleotide primers to provide a population of mutagenized DNA molecules.

6. (Original) A method according to claim 4, wherein mutagenizing comprises the steps of:
- combining DNA comprising said target region with retroviral integrase and a first set of complementary oligonucleotide primers, said primers comprising (a) a recognition sequence for said retroviral integrase and (b) a recognition site for a type II restriction endonuclease, wherein said retroviral integrase mediates the insertion of said first set of complementary oligonucleotide primers to provide a population of mutagenized DNA molecules
- cutting said population of mutagenized DNA molecules with said type II restriction endonuclease to provide cut DNA; and
- ligating to said cut DNA a second set of complementary oligonucleotide primers comprising a sequence tag.
7. (Original) A method according to claim 5, wherein said sequence of interest comprises a gene encoding a protein.
8. (Original) A method according to claim 4, wherein said selective condition is growth of cells in media lacking a nutrient that is an intermediate in a metabolic pathway.
9. (Original) A method according to claim 8, wherein said population of mutagenized DNA molecules are cloned into a filamentous bacteriophage vector with regulatory sequences for expression of said sequence of interest.
10. (Original) A method according to claim 5, wherein said sequence of interest comprises a regulatory gene.
11. (Original) A method according to claim 10, wherein said selective condition is growth in media containing a cytotoxic agent, and said regulatory gene controls expression of a gene conferring resistance to said cytotoxic agent.
12. (Currently Amended) A method according to ~~one of claims 4 to 11~~, claim 4, whereby the absence of a band under said selective condition and its presence under non-selective conditions is indicative of a target region which is essential under said selective condition.

13. (Currently Amended) A method according to ~~one of claims 1-12~~, claim 1, wherein said genome is a haploid genome.

14. (Original) A method according to claim 13, wherein said haploid genome is a bacterial genome.

15. (Original) A method for identifying essential genes in a genome of a cell grown in non-selective conditions, said method comprising:

saturation mutagenesis of said genome by insertion mutagenesis, whereby an oligonucleotide sequence is inserted in the target regions of said genome such that a population of cells having at least 90% of said target regions insertionally mutated is obtained;

growing said population of cells under non-selective conditions to provide a non-selected sub-population of cells;

amplifying a target region from said non-selected sub-population of cells, using a first primer which hybridizes to a known end of said target region, and a second primer which hybridizes to said oligonucleotide sequence, said first and second primers constituting a primer pair capable of giving rise to an amplification of an extension product when said oligonucleotide sequence is inserted into said target region; and

assessing for the presence or absence of said first and second extension product, whereby the presence thereof is indicative of a non-essential gene, whereas the absence thereof is indicative of an essential gene.

16. (Original) A method for identifying essential genes in a genome of a cell comprising:

saturation mutagenesis of said genome by insertion mutagenesis, whereby an oligonucleotide sequence is inserted in the target regions of said genome such that a population of cells having at least 90% of said target regions insertionally mutated is obtained;

growing said population of cells under selective or non-selective conditions to provide a selected or non-selected sub-population of cells;

amplifying a target region from said sub-population of cells, using a first primer which hybridizes to a known first end of said target region, and a second primer which hybridizes to another known end of said target region, said first and second primers thereby constituting a first primer pair, giving rise to a first extension product, and a third primer which hybridizes to said oligonucleotide sequence, said third primer constituting a second primer pair with one said first or second primer, said second primer pair enabling the amplification of a second extension product; and

assessing for the presence or absence of said first and second extension product, whereby the presence of the first and second extension products is indicative of a non-essential gene, whereas the presence of the first extension product and the absence of the second extension product is indicative of an essential gene.

17. (Original) A method according to claim 16, wherein said genome is a haploid genome.

18. (Currently Amended) A method according to claim 16 ~~to 18~~, wherein insertion mutagenesis is carried out with a transposable element.

19. (Currently Amended) A method according to ~~one of claims 1-18~~, claim 1, wherein said amplification is carried out by the polymerase chain reaction.

20. (Original) A method for identifying a therapeutic target in a genome of a cell grown in non-selective conditions, said method comprising:

saturation mutagenesis of said genome by insertion mutagenesis, whereby an oligonucleotide sequence is inserted in the target regions of said genome such that a population of cells having at least 90% of said target regions insertionally mutated is obtained;

growing said population of cells under non-selective conditions to provide a non-selected sub-population of cells;

amplifying a target region from said non-selected sub-population of cells, using a first primer which hybridizes to a known first end of said target region, and a second primer which hybridizes to another known end of said target region, said first and second primers thereby constituting a first primer pair, giving rise to a first extension product, and a third primer which hybridizes to said oligonucleotide sequence, said third primer constituting a second primer pair with one said first or second primer, said second primer pair enabling the amplification of a second extension product; and

assessing for the presence or absence of said first and second extension product, whereby the presence of the first extension product and the absence of the second extension product is indicative of an essential gene and hence of an identification of a therapeutic target in said cell.